The respiratory tract in pigs and its immune system: a review

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ABSTRACT: The growing amount of information regarding mucosal immunology in animals resulted from a need to better understand the pathogenesis of diseases entering the body through mucosa surfaces, including the respiratory tract. The second reason for such studies is associated with a search for alternative ways of vaccine application, including delivery to the mucosa of the respiratory tract. This review provides a structural and functional description of the immune system of the pig respiratory tract.

Keywords: swine; histology; host defence; specific immunity; innate immunity

Contents

1. Characteristics of immune mechanisms of the respiratory tract

The respiratory tract or respiratory system – *apparatus respiratorius* – is the group of specialised organs whose specific function is to mediate the transfer of oxygen from the air to the blood and carbon dioxide waste from the blood to the air and to regulate body temperature. These functions are performed by tubular and cavernous organs which allow atmospheric air to reach lungs and pulmonary parenchyma where gasses are exchanged with the blood through respiratory epithelium, the endothelium of blood capillaries and basal membranes. The upper respiratory tract begins with sensory receptors that monitor the inspired air, and wet, warm mucosa that provides filtration and humidifies and warms the inspired air.

According to the above classification, two morphologically and functionally separated, but cooperating immune systems, protect the respiratory tract (Bienenstock and Clancy 1994; Pabst 1996; Brandtzaeg et al. 1999) as follows: the immune system of the respiratory tract included in the immune system of mucosae in common, and the immune system of pulmonary parenchyma regulated by the local immune system.

The respiratory tract represented by the upper respiratory tract of nasal cavities at the beginning, and the lower respiratory tract comprising a fine ramification of bronchioles at the end constitute

Supported by the Ministry of Agriculture of the Czech Republic (Grants No. MZe 0002716202 and No. QJ1210120), the Ministry of Education, Youth and Sports of the Czech Republic (AdmireVet; Grant No. CZ 1.05/2.1.00/01.0006-ED 0006/01/01), and the Czech Science Foundation (Grant No. 524/06/P455).

the first part of this system and are lined by mucosa that has all the attributes of mucosa. Its specific protection is ensured by immune functions common to the immune systems of all mucosa with their primary effectors being IgA antibodies (Morgan et al. 1980). Thus, the immunity is referred to as mucosal. A hypothesis on the common immune system of mucosa was advanced in the mid-1970's (Bienenstock et al. 1974; McDermott and Bienenstock 1979) and suggested that antigenic stimulation at any inductive site of the mucosal immune system results in a specific immune response characterized by IgA antibody production in all of the other mucosa included in this system. This is enabled by a special type of circulation of B lymphocytes activated by antigens at inductive sites. This generally accepted conception has been gradually changed in some aspects on the basis of experimental results. They particularly concern the fact that antigenic impulses at the inductive sites of certain mucosa induce immune responses of varying intensities in the other mucosal areas (Brandtzaeg et al. 1999). Inductive sites in the respiratory tract are concentrated in aggregates of lymphoid tissue adjacent to mucosal surfaces of the nasal cavity, nasopharynx, larynx, trachea and bronchi. Such sites are nose associated lymphoid tissue (NALT) in the nasal cavity, lymphoid tissues of the Waldeyer's ring in the nasopharynx, larynx and trachea associated lymphoid tissue (LTALT), and bronchus associated lymphoid tissue (BALT) in the site of ramification of bronchi. Lymphoid tissue NALT, LTALT and BALT are not present in all animal species. Nose associated lymphoid tissue present as paired lymphoid aggregates in the floor of the nasal cavity is well described and characterised in small laboratory animals. In contrast, no comparable aggregates have been found at these sites in farm animals. Isolated lymphoid nodules have been described in horses and sheep (Mair et al. 1987; Stanley et al. 2001) but have not yet been investigated in other farm animal species. In contrast to NALT, lymphoid tissues of the Waldeyer's ring are better developed in farm animals than in rodents. The lymphoid structure includes aggregates of lymphoid nodules – tonsils – and isolated ones. In cattle, sheep, pigs and horses, larynx-associated lymphoid tissue has been described on the *epiglottis*, in the *vestibulum laryngis* and on the *plica aryepiglottica* (Liebler-Tenorio and Pabst 2006). In the trachea, lymphoid nodules have been described in horses only (Mair et al. 1987). The last part of lymphoid tissue connected to the respiratory tract is BALT. BALT is usually found in rats and rabbits. In a number of other animal species and in humans, the intensity of antigenic stimulation determines whether it develops or not (Bienenstock et al. 1999). In swine, BALT is not present before birth and its development is crucially dependent on antigenic stimulation (Pabst and Gehrke 1990; Delventhal et al. 1992). The presence of lymphoid aggregates in the lymphoid tissue of the respiratory tract results in a locally limited induction of the immune response; its role is to prevent an extreme immune activation of mucosae usually concurrent with their hyper-reactivity. It is not a strategy of the organism as a whole to functionally destabilise respiratory tract mucosa or digestive tract mucosa by repeated inflammations (Bienenstock et al. 1999). Due to that fact, intact mucosa of the respiratory tract is not usually able to ensure antigenic presentation with a subsequent immune response. This excessively happens after the disruption of its integrity, in cases where an antigen penetrates into a deeper layer of respiratory tract mucosa; the immune response may then be induced by a net of dendritic cells which are abundant here (Holt et al. 1990). Consequently, dendritic cells migrate into regional lymph nodes, which become a site of local immune response instead of mucosa (van der Brugge-Gamelkoorn et al. 1986; Havenith et al. 1993). The need to keep the respiratory tract outside the main inductive branch of specific immune responses is the reason why above all non-specific immune mechanisms become significant in respiratory tract. They remove foreign particles including microorganisms from the respiratory tract without producing inflammations or immune responses. Some types of specific immune responses are of a immunosuppressive character (Sedgwick and Holt 1985; Holt and Leivers 1982). This type of specific immune response has not been substantiated yet. It is comparable to oral tolerance, which controls the immune response of the intestine. Biological function that inhibits the immune response is based on the suppression of hypersensitive responses in the respiratory tract; its failure plays a key role in the development of asthma and other types of immunopathological states.

Another important is the immune system of proper pulmonary tissue. The unit of lung structure consists of terminal bronchioles which branch further into a series of transitional airways, the respiratory bronchioles and alveolar ducts. These

passages finally terminate in dilated spaces called alveolar sacs, which open into the alveoli. The airways are pliable tubes lined by respiratory mucosa and containing variable amounts of muscle plus cartilage in the larger airways. Alveolar cavity is not lined by mucosa, but with respiratory epithelium; a small sub-epithelial layer of fibroelastic tissue and a net of blood capillaries (haematoalveolar barrier) are the underlying tissue. Alveolar macrophages are present in the lumen of pulmonary alveoli; those participate together with other cellular and humoral factors in the non-specific defence of lungs (Cruijsen et al. 1992; Pabst 1996). The humoral component of specific pulmonary defence is represented by the antibodies present in alveolar fluid; these are largely IgG antibodies. In undisturbed pulmonary parenchyma, the majority of these antibodies are produced locally and only a small proportion of them are passively transferred across the haematoalveolar barrier (Charley and Corthier 1977; Morgan et al. 1980; Nechvatalova et al. 2011). If inflammatory lesions develop in pulmonary tissue, penetration of antibodies through this barrier is markedly increased (Krejci et al. 2005). Locally produced IgG antibodies prevail in lungs, but lower amounts of IgA antibodies are also present (Bradley et al. 1976a). This type of proper pulmonary tissue protection is also designated as local type immunity. The cellular component of specific pulmonary protection (if the mentioned immunoglobulin-producing plasma cells are not considered) is mostly represented by T lymphocytes (Pabst and Binns 1994; Pabst and Tschernig 1995; Pabst 1996).

2. Immune mechanisms and morphology of the respiratory tract of pigs

The morphology of the respiratory tract of pigs has been well described. Particular attention was also paid to common immune mechanisms of mucosal immunity including the immune system of the respiratory tract (Breeze et al. 1976; Reynolds 1991). The detailed knowledge necessary for gaining a complete picture of the immune system of the respiratory tract of pigs has not been obtained yet despite the fact that the immune system of the respiratory tract of pigs has been the focus of intense research (Bradley et al. 1976a,b; Charley and Corthier 1977; Euzeby 1993; Pabst and Binns 1994; Pabst and Tschernig 1995; Pabst 1996; Bailey and Stokes 1998). The present review describing the immune mechanisms of the respiratory tract is based on natural relationships and mechanisms described for other animal species with an emphasis on known differences typical for pigs.

2.1. The respiratory tract

2.1.1. Morphology of the respiratory tract

The respiratory tract is divided anatomically into two distinct parts, the upper and the lower respiratory tracts, which are separated by the pharynx. The upper respiratory tract comprises the nasal cavity and the nasopharynx. The lower respiratory tract begins with the larynx and then continues into the thorax as the trachea, bronchi, bronchioles and lungs. Cartilage or bone provide a supporting skeleton for the wall of the airways and prevent the collapse of these airways during respiration.

Air enters the respiratory tract through small round nostrils and a pair of **nasal cavities** (*cavum nasi*) situated in the external nose (*nasus externus*), which is designated the snout (*rostrum*) in pig. Nasal cavities are lined by pseudostratified ciliated columnar epithelium containing lymphoreticular tissue aggregates, loose lymphocytes and combined tuboalveolar glands. Air passes through the nasopharyngeal tube into the pharynx where the airways stand at the intersection with the digestive tract. The **respiratory part of the pharynx** (*nasopharynx*) is lined by mucosa covered with a pseudostratified ciliated columnar epithelium and goblet cells. *Lamina propria mucosae* comprises combined fine glandules and is abundant in infiltrated lymphocytes in the form of isolated lymphatic nodules or tonsils, which are a component of the Waldeyer's ring (*tonsilla veli palatini*, *t. pharyngea*, *t. tubaria*, *t. paraepiglotica* and *t. lingualis*); place important for inducing immunity at mucosal sites (Liebler-Tenorio and Pabst 2006). The lower respiratory tract begins with the **larynx** (*larynx*) and links the nasopharynx with the lower respiratory tract. The larynx contains epiglotis and vocal cords (*plicae vocales*). The mucosa of the larynx (except for epiglotis and vocal cords) is lined by a pseudostratified columnar epithelium and isolated goblet cells; in the submucosa of the *epiglottis*, *plicae aryepiglotticae* and *vestibulum laryngis*, there is an accumulation of lymphatic tissue in the form of lymphatic nodules.

The **trachea** (*trachea*) of pigs consists of 32–36 C-shaped rings of hyaline cartilage; the gaps between the rings are bridged by strong fibroelastic membranes. The trachea passes ventrally along the neck through the *apertura thoracis cranialis* into the thoracic cavity; it repeatedly ramifies at the level of the heart into smaller and smaller airways (*bronchi* and *bronchioli* in the lungs). The trachea is lined by ciliated pseudostratified columnar epithelium and isolated goblet cells comparable to those in extrapulmonary bronchi. The mucosa – *lamina propria mucosae* – contains small islets of lymphoreticular tissue and combined tuboalveolar glands.

The **lung** (*pulmo*) is a paired organ of respiration lying on either side of the heart, within the thoracic cavity. In pigs, the right lung is divided into four lobes – *lobus cranialis*, *medius*, *caudalis* and *accessorius* and the left lung is divided into two lobes– *lobus cranialis* (*pars cranialis* and *caudalis*) and *lobus caudalis*. Lung lobes are further divided into smaller areas, lung segments, which are morphologically and functionally independent pulmonary tissues divided into pulmonary lobules – *lobuli pulmonis,* the smallest ones being terminal bronchioles (*bronchioli terminales*).

Lungs lie in the thoracic cavity whose walls are lined by parietal pleura (*pleura parietalis)*; that transits as pulmonary pleura (*pleura pulmonis*) to the lung surface. The l**ymph nodes** (l. n.) of the thoracic cavity are concentrated into four lymphoid centres; two of them are parietal l. n. (*lc. thoracicum dorsale* and *lc. thoracicum ventrale*) and two visceral l. n. (*lc. mediastinale* and *lc. bronchale*). Lymph from thoracic cavity organs such as the pharynx, trachea, thymus, heart and lungs is conducted away via visceral centres. The *lymphocentrum bronchiale* in pigs consists of lymph nodes adjacent to the bronchi (*lnn. tracheobronchales dextri*, *sinistri et medii).*

2.1.2. Non-specific immune mechanisms of the respiratory tract

A highly complex and effective system of non-specific immune mechanisms that can eliminate more than 90% of the microorganisms that penetrate the airways is present in the respiratory tract. This non-specific immune system plays a key role in preventing infection without excessive activation of a specific immune response; despite this, some of its components form transitional links between the specific and non-specific immune responses.

Mucosa and its secretions play an essential role in the non-specific defence system of the respiratory tract. Mucosa is lined by epithelium, which does not only form a mechanical barrier, but also shows significant kinetic and secretory activities. Together with produced mucus, mucosa forms a system designated as the mucociliary apparatus.

The **mucociliary apparatus** is one of the most significant systems essential for the protection of respiratory mucosa. Its primary role is to continuously remove xenobiotic particles (incl. dust particles and pathogenic microorganisms that have penetrated the airways) from mucosa. Due to its extent of coverage, the mucocilary apparatus represents a barrier for these pathogens that is almost unsurpassable under physiological conditions. The clearance function of respiratory tract mucosa results from a mutual interaction between ciliary epithelium and the uppermost layer of mucus.

The **ciliary epithelium** covers a major part of the mucosal surfaces of the respiratory tract from the nasal cavity to proximal parts of terminal bronchioles where it transits to columnar non-ciliated epithelium (Baskerville 1970a). Ciliary epithelium consists of ciliary epithelial cells designated as ciliary cells. Their shape is columnar in the proximal part of the respiratory tract and becomes cuboidal with shorter ciliae in the caudal part. Ciliary columnar cells are about 20 μm in height and 7 μm in width; they are narrower towards the base. The surface of each of the cells is equipped with approximately 200–300 ciliae and their beat frequency is 300–600 per minute (Breeze et al. 1976; Reynolds 1991). Each cilium is about 5 μm in length and 0.25 μm in width. Microvilli from ciliary cells measuring approximately 0.7 μm in length have been described (Baskerville 1970b); however, their function has not yet been well characterised. Accordingly, they are viewed as a marker of the immaturity of epithelial cells. Microvilli (2 μm in length and 0.17 μm in width) have been usually detected on the so called brush cells that are other columnar elements in the epithelium; they are particularly found in higher numbers in proximal bronchioles in pigs (Baskerville 1970a), where they play an important role in the absorption of abundant secretions from the respiratory tract (Reynolds 1991). Due to the fact that they contain afferent nerve endings on their basal surface, they are also considered as sensory receptors.

Epithelial cells in the respiratory tract play a role not only as a mechanical barrier on the border

between the external environment and internal tissues but are involved in the first reaction to invading pathogens: the induction of inflammation, and recruitment of phagocytosing cells (Kato and Schleimer 2007; Bartlett et al. 2008; Gomez and Prince 2008; Gon 2008).

Mucus containing dust particles and trapped microorganisms is removed by ciliary activity. The mucosal component of mucus is transported along its basal, serous layer proximally towards the pharynx where mucus is swallowed or coughed up in the case of its overproduction. With respect to immunology, the swallowing of mucus is very important because it ensures continuous oral immunisation of the intestines with low doses of live or partly digested microorganisms, which have entered the organism aerogenically from the external environment. With regard to mucosal immune response activation, induction via Peyer's patches in the intestines is more significant than that via similar structures in bronchial walls (BALT). Mucus present in small bronchioles moves slowly (1–2 mm/min); its speed increases when moving in the direction of the mouth, and reaches up to 20 mm/min in trachea (Cone 1999).

The mucus is one of the significant constituents of the mucociliary apparatus. It is a type of secretion that is mediated by secretory cells along the entire respiratory tract. However, its composition differs in different sections of the respiratory tract. A mucous character of this secretion prevails in the respiratory tract (proper mucus), whilst its character is serous in terminal bronchi and in lung alveoli (Cone 1999). Secretion serves a multitude of defence functions: besides the functions associated with the respiratory activities of the respiratory tract surface it plays a series of significant defence roles. It forms a dynamic mechanical cover for mucosal epithelium, and is a medium containing high levels of non-specific antibacterial substances (Ganz 1999; Travis et al. 1999; Zhang et al. 2000; Schutte and McCray 2002).

Mucus is structured into two layers differing in composition and viscosity. The upper thin layer is a sticky and slippery gel with viscoelastic properties; its matrix is composed of glycoprotein molecules of mucin (approximately 1–3%). This upper (mucin) layer moves, and floats on the lower layer; the latter may often be serous in nature, with moving cilia. The layer of mucus blanket lining the respiratory tract is overall quite thin (in comparison with the similar cover of the gastrointestinal mucosa) and in fact does not overlap the tops of cilia by more than 2 μm (Cone 1999). The secretion of each mucus constituent is affected both by endogenous mechanisms which are controlled by vegetative nerves, and by a number of exogenous factors. Their activity may seriously disturb the defence function of the mucosal barrier of the respiratory tract.

Accordingly, the proper function of the mucociliary system does not only depend on ciliary epithelium activity, but also on finely balanced secretions of serous and mucinous constituents of mucus. The viscosity of the lining (mucin) layer depends on the mucin/water ratio and on the presence of other substances (lipids, proteins and ions). Physical properties of the mucous layer become a protective barrier, which prevents microorganisms from penetration the lower respiratory tract or the mucosal epithelium. Mucin itself, due to its specific properties, forms a physical-chemical barrier preventing microorganisms from binding to superficial structures of epithelial cells. The shape of a mucin molecule resembles a bottle brush; the molecular axis is a protein chain with a number of lateral oligosaccharidic chains. Their composition is comparable to oligosaccharidic structures on the surface of epithelial cells (*glycocalyx*), which are target sites for microorganisms that bind to their surfaces. Mucin thus produces surplus binding sites; these are preferably offered to penetrating microorganism and function to induce a type of a competitive binding (Cone 1999). Subsequently, the mucin-bound microorganisms are removed by the mucociliary apparatus from the respiratory tract. Thus, e.g., the bacterium *Mycoplasma hyopneumoniae* is eliminated from the pig airways in this way (Zhang et al. 1994). IgA antibodies are a significant component of the mucosal layer; they are secreted into this layer across mucosal epithelial cells. The mucosal layer is the primary carrier of antibodies of this isotype because more of them are secreted into this layer than into blood circulation (Mestecky et al. 1986). The origin and distribution of antibodies of this isotype will be described below.

Respiratory tract secretion is achieved by secretory cells of different types. Mucus present in the proximal part of the respiratory tract is secreted by goblet cells scattered among epithelial cells (their average ratio is about 1 : 5; they increase in number in the case of chronic inflammation) and they release their secretions straight into the lumen of the respiratory tract (Reynolds 1991). These cells become

scarcer distally and they are not present in terminal bronchioli. They are replaced there by Clara cells; their secretions are of serous character and contain proteins, lipids, phospholipids, lecithin and other superficially active substances. The marginal line of goblet cells is shorter distally than that of ciliary epithelium. This arrangement does not allow mucus to gather in the respiratory part of the airways. The cells of combined glandules present in the *lamina propria mucosae* and submucosal layer of the respiratory tract is another producer of secretion. Their secretion are produced by two cell types: mucous and serous, and it is transported to the mucosal surface through a short duct. The number of secretory epithelium cells in glandules manifold exceeds the number of goblet cells (Reynolds 1991). Accordingly, it is assumed that these glandules play a more significant role in secretory activity.

Despite the fact that the mucociliary apparatus can eliminate large numbers of microorganisms, it may be partly or completely paralysed or damaged by certain pathogens. Many bacteria possess defence mechanisms to help them overcome this non-specific constituent of respiratory tract immunity that might prevent their colonisation and propagation in the respiratory tract. Damage to cilia is most common, either by immobilisation or more usually by damage, or suppression of mucin production. This effect was very well documented in pigs after their infection with bacterium *Mycoplasma hyopneumoniae*. This bacterium does not only cause destruction to ciliae in vast areas of the ciliary epithelium, but also participates in decreasing mucin production (DeBey et al. 1992; DeBey and Ross 1994). Some other pathogenic microorganisms that are causative agents of respiratory diseases may damage epithelial cell cilia. These are above all *Pasteurella multocida* (Kamp and Kimman 1988) and *Bordetella bronchiseptica* (Yokomizo and Shimizu 1979). However, *Bordetella bronchiseptica* affects epithelial cells of the nasal cavity and with some exceptions does not tend to spread to the lower respiratory tract. Superficial adhesion of these pathogens is a precondition for the destructive activity of microorganims on ciliary epithelium. Its mechanism has not yet been fully clarified. Recently, the interaction between the surface of cilia and membrane proteins on the surface of *Mycoplasma hyopneumoniae* has been intensively studied (Minion et al. 2000). The damage of cilia by pathogenic microorganisms may be accomplished by the action of necrotoxins, such as in the case of *Pasteurella multocida* (Kamp and Kimman 1988), or proteolytic enzymes such as in the case of *Pseudomonas aeruginosa* (Hingley et al. 1986), or other less investigated mechanisms. It has not yet been elucidated by what mechanisms microorganisms penetrate the mucus layer. The penetration may be facilitated by proteolytic enzymes that disturb the compactness of the mucosal gel. One of the significant factors in the pathogenicity of some microorganisms is their capability of penetrating the ciliary epithelium, and the adherence to cilia with subsequent destruction. Not only microbial activity disturbs the mucociliary apparatus; also a number of other factors from the internal or external environment (drugs, atmosphere, toxic and nutritional factors) can mediate such effects. Once disturbed, the penetration and adherence of other pathogens usually occurs. These do not possess the necessary pathogenic factors and would be eliminated from an organism under physiological conditions without having the chance to seriously harm the host. Super-infections commonly seen in animal herds follow these circumstances.

As mentioned above, respiratory tract secretions contain a number of substances with considerable antimicrobial activity. The following are most significant: lactoferrin, defensins and lysozyme. The presence of these and some other substances with antimicrobial activity in the mucosal secretions of pigs was documented by Zhang et al. (2000).

Lactoferrin – is a protein found in specific granules of neutrophils; it is structurally and functionally closely related to serum transferrin. Lactoferrin is released into secretions of the airways from both neutrophils and secretory epithelial cells. Its bactericidal activity is exerted through attracting and binding ferric ions that are essential for the growth of the majority of pathogenic bacteria (Euzeby 1993; Pruitt et al. 1999; Adlerova et al. 2008).

Defensins are relatively abundant in secretions of the respiratory tract. Research has recently focused on their antimicrobial activities. In contrast to intestinal mucosa, where in particular defensins-α are present, defensins-β prevail in the respiratory tract. Both branches of defensins are small peptides (3–5 kDa) that show considerable antimicrobial activity (Zhang et al. 2000). They are effective broad-spectrum antimicrobials involved in the defence against bacterial, fungal and viral agents. Epithelial cells of the mucosa and myeloid cells (particularly neutrophil granulocytes) may constitutively express defensins. However, much higher levels of

defensins are produced during infection. Their production is induced by inflammatory as well as anti-inflammatory cytokines, growth factors and microbial products (Schutte and McCray 2002; Hiemstra 2007). Defensins increase the permeability of bacterial membranes. Besides bactericidal properties, defensins may play other roles; they exert chemoattractive activity for immature dendritic cells, monocytes and T lymphocytes (Ganz 1999; Schutte and McCray 2002).

Lysozyme is an enzyme produced by the majority of phagocytosing cells and to a lesser extent by epithelial cells. It is particularly involved in the process of digestion of cell walls of Gram-positive bacteria (it hydrolyses a specific glycosidic bond in N-acetylglukosamine and N-acetylmuramic acid) and is the most common bactericidal enzyme naturally occurring in the secretions of the respiratory tract in the majority of mammals (Travis et al. 1999).

Interferons participate in the non-specific defence activity of the respiratory system, similarly as other organ systems; in the case of inflammation and penetration of complement constituents from the peripheral blood into the respiratory tract mucosa a **complement cascade** process may be triggered (Euzeby 1993). Interferons-α and -β are produced mainly by virus-infected cells. Interferons bind to receptors present on both infected and non-infected cells and induce an "antiviral state", i.e., they induce the synthesis of enzymes with activity against viral replication. Interferon-γ is a product of antigenically-specific Th1 cells and NK cells and controls various aspects of immune responses. Interferons are active as inflammation inducers, activators of macrophages, mast cells and fibroblasts, and as differentiation factors for Th1 cells. Interferons are essential for the suppression of intracellular infections (Euzeby 1993).

2.1.3. Specific immune mechanisms of the respiratory tract

The **humoral component of the specific immune defence** of the respiratory tract of pigs comprises predominantly IgA and IgG antibodies, and to a lesser extent IgM antibodies. Polymeric forms of IgA antibodies, usually dimers, are present in secretions; these are of secretory type (sIgA). IgM antibodies may also be of secretory type (sIgM). sIgA (sIgM) antibodies present on respiratory tract mucosa are synthesised locally. The vast majority of sIgA are produced by non-organized aggregates of B lymphocytes in various stages of differentiation up to plasma cells that are diffusely scattered throughout the *lamina propria* of respiratory tract mucosa (Bradley et al. 1976a; Morgan et al. 1980). A part of these antibodies may be produced by organised aggregates of lymphoid tissues including tonsils (Bradley et al. 1976b). The presence of B lymphocytes on mucosa, and the specific character of antibodies produced by plasma cells results from recirculation of activated B lymphocytes within the common immune system of mucosa. After antigenic induction, specific B lymphocytes that are partly differentiated migrate through lymph vessels to local lymph nodes and then to blood circulation; subsequently, they pass through the endothelium of postcapillary venules and preferentially settle in the diffused lymphoid tissue of mucosae (their usual return to their place of origin has been designated as "*homing*"). The role of different adhesive molecules, incl. α4β7 and α1β7, which interact with VCAM-1 and MadCAM has been described (Bourges et al. 2007).

Returning B lymphocytes perform their function within mucosa: they differentiate into IgA-producing plasmatic cells. IgA antibodies penetrate the epithelial layer in a way typical for secretory types of antibodies (Mostov and Kaetzel 1999). IgA bind transport receptors on the basement membrane of epithelial cells; the complex of transport receptor and IgA undergoes endocytosis and is transferred by transcytosis to the opposite side (lumen) of the cell where it fuses with the membrane; a part of the receptor molecule (secretory component) with bound IgA is digested by proteolysis. IgA usually penetrate the secretory epithelium of combined bronchial glandules through to the mucosal surface (Bradley et al. 1976a). Up to 97% of all IgA antibodies present in the airways of pigs penetrate through to mucosal surfaces in this way (Morgan et al. 1980). The origin of IgG antibodies in the airways of pigs is not uniform. A part of them is produced locally (Bradley et al. 1976a; Morgan et al. 1980); however, the majority of them penetrate through to the airways along with alveolar fluid where by diffusion from blood circulation they traverse a fine pulmonary hematoalveolar barrier (Reynolds and Merrill 1981). In the case of pulmonary inflammations, this diffusion increases manifold. The penetration of colostrum antibodies through to nasal and bronchial mucosa

has been described in newborn piglets (Mensik et al. 1971a, b; Bradley et al. 1976a; Nechvatalova et al. 2011). However, antibodies circulating in blood do not penetrate through to undamaged mucosa of the airways in older age piglet categories and in adult pigs (Charley and Corthier 1977).

Their ratio of secretions from different parts of the respiratory tract is consistent with the described origin of major antibody isotypes. The results of Holmgren et al. (1973) and Morgan et al. (1980) confirmed a predominance of IgA antibodies in secretions of proximal parts of the respiratory tract. The IgA/IgG ratio is balanced or reversed in secretions from the most distal parts of the airways. Their functions are consistent with the described rates of respective antibody isotypes on mucosal membranes. The function of polymer secretory IgA antibodies that prevail in the parts lined by mucosa is to protect the respiratory system from the activity of microorganisms that penetrate through to the respiratory system and do not induce an inflammatory process. These antibodies are difficult to digest by proteases and therefore they become significant defence factors protecting mucosal surfaces. IgA antibodies prevent the adhesion of microorganisms to mucosal surfaces and in a concerted action with other non-specific defence mechanisms (mucus, mucociliary apparatus, antibacterial systems) they facilitate their elimination from the organism. IgA slightly opsonize; therefore, antigen – IgA complexes can bind Fc-receptors of effector cells and may be removed by phagocytosis. IgA antibodies do not activate the complement pathway, but pathogenic microorganisms are nevertheless neutralised, aggregated and subsequently eliminated. No inflammatory response or mucosal damage is induced (Russell et al. 1999). It is not the strategy of a mucosa intensively exposed to antigens from the external environment to respond to each stimulus with inflammation. However, IgG antibodies do activate complement; they are also effective as opsonins and facilitate the phagocytosis of immunocomplexes originating from the binding of these antibodies to the surfaces of microorganisms.

The **cell component of the specific defence** of the respiratory tract comprises lymphoid tissue cells present in the mucosal *lamina propria* and adjacent regional lymph nodes. The proper lymphoid tissue of respiratory mucosa includes two morphologically and functionally distinct parts. The non-organised lymphoid tissue is the larger of them; it is composed of a higher number of cells and is usually active as an effector. The organised lymphoid tissue is a site of immune response induction. The organised lymphoid tissue forms aggregates comparable to lymph nodes in certain parts of the airways; these directly adhere to mucosal surfaces. In contrast to lymph nodes, these formations possess neither a strong ligamentous envelope nor afferent lymph vessels.

Non-organised lymphoid tissue of the respiratory tract mucosa is composed of diffusely scattered lymphocytes and rather small clusters of lymphocytes. Plasma cells, B lymphocytes and T lymphocytes prevail in these non-organised clusters (T lymphocytes with superficial $CD4^+$ antigen prevail in *lamina propria)* (Pabst 1996). The ratio of these cells depends on antigenic pressure or on the pathological process in the airways (Puci et al. 1982). Besides the above mentioned cell types, macrophages and to a lesser extent granulocytes and mast cells are present in this lymphoid tissue. Intraepithelial lymphocytes (IEL) were also found in the epithelial layer of respiratory tract mucosa, which is comparable to epithelium of other mucosae (Fournier et al. 1989; Hameleers et al. 1989). While investigating these cells in humans it was found that these were lymphocytes mostly expressing αβ T-cell receptor (αβTCR). The presence of IEL was described in the intestinal epithelium of pigs; we assume that the population of these cells is also present in the respiratory tract. In new-born piglets, IEL do not possess the surface antigens CD2, CD4 and CD8 (these are $CD2^-$, $CD4^-$, $CD8^-$); they begin to express surface antigen CD2 in the first weeks of life, and they also express surface antigen CD8, and thus possess $CD2^+$, $CD8^+$ from week 7 on (Whary et al. 1995). Despite the fact that $CD8^+$ lymphocytes generally prevail over $CD4^+$ lymphocytes, the dominance of $CD8^+$ lymphocytes is less pronounced in bronchial epithelium ($CD4^+/CD8^+$ ratio is approximately 0.4) in comparison with intestinal epithelium ($CD4^+/CD8^+$ ratio is approximately 0.1) (Goto et al. 2000). IEL is thought to participate in mucosal immunoregulation, particularly in the maintenance of mucosal integrity (Erle and Pabst 2000).

Dendritic cells (DC) are a distinct, quite recently described cell population involved in the immune activities on mucosae (Sertl et al. 1986; Holt et al. 1989). The phenotypic characteristics of two respiratory tract DC populations (myeloid dendritic cells – mDCs and plasmacytoid dendritic cells – pDCs) have been described in the mouse and hu-

man. These cells lack expression of lineage-specific markers (incl. CD3, CD19, CD20, CD14, CD16 and CD56) and mDCs and pDCs can be discriminated based on CD11c and CD123 expression, respectively. Both subsets of DCs have been described also in swine (Summerfield and McCullough 2009). These cells are situated on the basement membrane of epithelium where they form a dense interconnected net that increases in density after birth (von Garnier and Nicod 2009). These cells are capable of mannose receptor-mediated antigen endocytosis and subsequently they present this antigen. It seems that they play a key role in the induction of an immune response by respiratory tract mucosa, particularly in animal species with not well organised lymphoid tissue (Reynolds 1991; McWilliam et al. 1994). Airway mucosal DCs continuously sample incoming airborne antigens by extending their dendrites through the intact epithelial layer into the airway lumen (von Garnier and Nicod 2009). Their participation in mucosal immune response regulation and in immune response control towards Th1 or Th2 is significant (Holt 2000). The cells of the non-organized lymphoid tissue of the respiratory tract in pigs were previously a focus of attention (Bradley et al. 1976a, b); however, more recent publications are scarce. The above mentioned authors succeeded in the detection and description of immunoglobulin-containing cells. These are plasma cell-like cells and are usually aggregated around mucosal glandules. They produce IgA immunoglobulins; however, the proportions of IgG and IgM immunoglobulin-containing cells were also significant. These immunoglobulin-producing cells were not detected on the respiratory tract mucosa of new-born piglets. They began to appear between day 5 and 7 of life and reached numbers comparable with adult animals at approximately one month of life. Despite the fact that data confirming the presence of dendritic cells and intra-epithelial lymphocytes in the respiratory tract of pigs is scarce, we assume that these cell populations are present in this animal species.

Organised mucosa-associated lymphoid tissue (MALT) is strategically located at different sites to allow efficient antigen sampling from mucosal surfaces. Based on the anatomical localisation, MALT structures of the respiratory tract can be subdivided into nose-associated lymphoid tissue (NALT), lymphoid tissues of Waldeyer's ring, larynx-associated lymphoid tissue (LTALT) and MALT which is present at the site of bronchial ramification as bronchus-associated lymphoid tissue (BALT) (Liebler-Tenorio and Pabst 2006). This organised lymphoid tissue is specialised in the recognition and processing of antigens, and in the primary activation of B lymphocytes. These aggregates of organised lymphoid tissue resemble Peyer's patches of ileum and both are involved in the mucosal immune system, playing a key role in this immune system. They are the sites of mucosal immune response induction (Bienenstock et al. 1999). Antigens are sampled from the mucosal surface and cognate naive B- and T lymphocytes are then stimulated. MALT structures are the origin of lymphocyte trafficking to mucosal effector sites. MALT contains lymphatics which transport immune cells and antigens to regional lymph nodes that can therefore be called part of the inductive sites of mucosa and augment the immune responses (Liebler-Tenorio and Pabst 2006).

BALT does not develop in all pigs and some other animal species such as mice, cats, dogs, humans, primates. It only develops after contact with pathogens, hence usually after an infection of the respiratory tract (Jericho, 1970; Delventhal et al. 1992). Provided BALT develops in pigs, its structure and in particular its topography differ in certain aspects from those in the other animal species studied to date. The T- and B-lymphocyte-containing areas are not as clearly separated from each other as is the case in other animal species (Delventhal et al. 1992); BALT in pigs is usually localised at the site of ramification of the bronchioles and to a lesser extent in small, cartilage non-supported bronchi (Huang et al. 1990). Due to the fact that BALT occurs non-constitutively in some animal species, the essential role of BALT in the induction of mucosal response in respiratory tract is disputable. This role may be taken over by the above mentioned dendritic cells. Besides BALT, other submucosal areas of organised lymphoid tissue are present in the upper respiratory tract; these are similar in structure and function. The lymphoid tissue designated as tonsils is aggregated around the upper respiratory tract at the site where it intersects with the digestive tract. These small lymphoid organs are present in great numbers in the nasopharyngeal mucosa, oral part of the pharynx, soft palate and tongue. Their topographic arrangement as a continuous lymphoid pharyngeal ring is designated as the Waldeyer's lymphoid pharyngeal ring. Despite the fact that this term is also used in animals, e.g., for anatomic description of these lymphoid tis-

sues, the term Waldeyer's lymphoid pharyngeal ring is mainly used in literature in human studie. In animals, these tissues are described as nasal-associated lymphoid tissue (NALT) (Kuper et al. 1992, 2003). With respect to the comparable function and morphology of BALT and NALT, these two systems are not discriminated in this review, although it seems that BALT and NALT are two distinct systems (Brandtzaeg et al. 1999).

2.2. The proper pulmonary tissue (pulmonary lobules)

Two separate immune systems are distinguished within the respiratory tract in the present study. Accordingly, respiratory bronchioles, alveolar ductules and air sacks, i.e., the part of the lungs lined with respiratory epithelium, are considered as the proper pulmonary tissue.

2.2.1. Morphology of the pulmonary tissue

Respiratory epithelium undergoes a progressive transition from pseudostratified columnar ciliated form in the larynx and trachea to a simple, cuboidal, non-ciliated form in the finest airways.

Pulmonary lobes constitute the proper lung tissue where stratified columnar epithelium transits into simple non-ciliated columnar epithelium and simple flattened epithelium is found in alveolar ductules (*ductus alveolares*). The alveolar cavity is lined by respiratory epithelium consisting of two types of pneumocytes: membranous pneumocytes (type I) and larger granulated pneumocytes (type II). Different populations of macrophages are present in the lumen and pulmonary tissue as well.

2.2.2. Non-specific immune mechanisms of the pulmonary tissue

The **humoral constituent of non-specific pulmonary defence** is alveolar fluid; its major constituent is pulmonary surfactant that contains superficially active substances: a phospholipid film with a protein-polysaccharide layer that decreases tension on epithelial surfaces. The surfactant does not only facilitate respiration and prevent lung collapse in expiration, but also contains a series of agents which are essential for the non-specific defence of pulmonary tissue. The best recognized substances are two proteins from a group of agents designated as collectins. These are surfactant protein A and surfactant protein D (SP-A a SP-D). Besides the surfactant proteins, conglutinin- and mannose-binding proteins are also classified as collectins. Collagen sequences are common to the proteins of this group (similarly as in C1q, which is a constituent of the complement). Despite the fact that surfactant proteins are not capable of opsonisation and do not activate complement, they markedly increase clearance, accelerate phagocytosis and increase the bactericidal activity of phagocytosing cells (Crouch 1998; Hermans and Bernard 1998, Haagsman et al. 2008).

The **cellular constituent of non-specific pulmonary defence** is represented by a wide net of macrophages and dendritic cells. They are classified according to their localisation as alveolar, interstitial and intravascular macrophages. Alveolar macrophages with increased capability of ingestion are found in the alveolar lumen; due to that fact, their phagocytic function predominates (Chitko-McKown and Blecha 1992; Cruijsen et al. 1992; Marriot and Dockrell 2007), in contrast to pulmonary intravascular macrophages with primary cytolytic function. Recently, the mechanism involved in the hyporesponsiveness of alveolar macrophages has been described. The interaction between the molecules CD200 and CD200R seems to be important for the attenuation of inflammatory response. These molecules were detected also in the respiratory tract of intact animals and their expression declined after endotoxemia (Hoek et al. 2000; Jiang-Shieh et al. 2010). In healthy animals, intravascular macrophages adhere to blood vessel endothelium; however, during inflammation, they promptly penetrate through to affected sites and remove cellular and non-cellular residues from both the site of inflammation and blood (Bertram 1986). The density of the net of intravascular macrophages is not the same in all animal species. It is very high in pigs, sheep, goats, cattle and cats and lower in other animal species (Pabst and Binns 1994; Pabst 1996). Intravascular macrophages replace the function of hepatic macrophages in the above mentioned animal species (Kupffer cells) and the lungs are the organ responsible for their blood clearance in these animals (Crocker et al. 1981; Pabst 1996). Intravascular macrophages gradually increase in number during postnatal development. They cover only 2% of capillary surface in new-born piglets;

however, 16% of surface is covered at the age of about one month (Winkler and Cheville 1987). The production of anti-inflammatory cytokines is comparable in both populations of macrophages. Due to the fact that macrophages are present in the alveolar lumen they prevail in bronchoalveolar lavages (BALF); these include more than 90% of all nucleated cells in the BALF of healthy animals (Nechvatalova et al. 2005). Nearly all alveolar macrophages in healthy pigs are of $CD14^+$, $CD163^+$, $CD203\alpha^+$ and $MHCII^+$ phenotype (Ondrackova et al. 2010).

Lymphocytes, dendritic cells and neutrophil granulocytes are present in much lower numbers in BALF. Dendritic cells are found in the alveolar space, the alveolar epithelial layer, and the interstitium (von Garnier and Nicod 2009). Granulocyte numbers in BALF are increased in cases of bacterial infections. For example, a strong influx of granulocytes into the BALF is associated with *Actinobacillus pleuropleumoniae* infection (Nechvatalova at al., 2005).

2.2.3. Specific immune mechanisms of the pulmonary tissue

The **humoral constituent of specific pulmonary defence** is represented by antibodies present in the alveolar fluid or in blood circulating through pulmonary vessels. These antibodies are mostly IgG immunoglobulins. Most of these antibodies are produced locally in non-damaged lungs, and only a small proportion penetrates passively across the haematoalveolar barrier (Charley and Corthier 1977; Morgan et al. 1980). The penetration of immunoglobulins across the haematoalveolar barrier significantly increases when pulmonary inflammatory lesions are present (Krejci et al. 2005). It is again above all the penetration of IgG immunoglobulins that function as opsonins. The follow-up complement activation in the alveoli can cause a problem; however, the induction of a rapid and sufficiently intensive inflammatory response is a desirable defence mechanism. It contributes to a rapid inactivation of pathogenic microorganisms and accelerated exudation associated with an intensive efflux of phagocytosing neutrophil granulocytes and other IgG antibodies from blood circulation. Intensive inflammation limited by time and place is an essential defence mechanism. Besides the above mentioned IgG antibodies, IgA antibodies are also produced in lungs to a lesser extent. This was confirmed by Bradley et al. (1976a) who found relatively numerous aggregates of IgA antibody-producing plasma cells in the lung interstitium.

The **cellular constituents of specific pulmonary defence** are mostly T lymphocytes (if the above plasma cells are not considered). These lymphocytes may be classified into several categories based on their localisation according to Pabst and Binns (1994) and Pabst (1996). They categorised lymphocytes according to the site of their presence as follows: interstitial lymphocytes, lymphocytes present in the intra-alveolar space of pulmonary tissue, and intravascular lymphocytes.

Interstitial lymphocytes represent numerous subpopulations of lymphocytes. Their count in adult pig lungs is up to 1×10^{10} (Pabst and Tschernig 1995). T lymphocyte counts prevail (more than 70%) over B lymphocytes (10%) and NK cell counts. Among T lymphocytes, $CD4^+$cells prevail over $CD8^+$ cells. The frequent occurrence of CD45RO is noteworthy (Pabst and Tschernig 1995; Pabst 1996). These lymphocytes usually penetrate from blood circulation into the interstitium. Pabst and Binns conducted experiments with labelled lymphocytes (Binns and Pabst 1994; Pabst and Binns 1994), administering them intravenously either to the pulmonary artery (*arteria pulmonalis*) or aorta. They detected high amounts of these labelled lymphocytes in the interstitium after a short time, irrespective of the vessel of administration. When lymphocytes are administered into the aorta, they have to pass through the entire vascular system of an organism. The purpose of this migratory activity has not yet been understood.

Lymphocytes present in samples obtained by bronchoalveolar lavages of intra-alveolar space comprise about 2–10% of nucleated cells. Their approximate number is comparable with the percentage of interstitial lymphocytes (5%) and all circulating lymphocytes (about 5%) (Holt et al. 1986). The majority of the lymphocytes are T lymphocytes; cells with the surface antigen $CD4^+$ ($CD4^+/CD8^+$ ratio is approximately 1.7) prevail. B lymphocytes comprise about 5–10% of the total lymphocyte pool in this compartment (Holt et al. 1986). A strikingly high percentage of lymphocytes that express the CD45RO marker (a characteristic feature of memory cells) are present among lymphocytes in the intra-alveolar space. Lymphocytes found in the bronchoalveolar space do not seem to be in their terminal stage of development. After experimental administration of labelled lymphocytes into bron-

chi, a proportion of them were detected in regional lymph nodes after 24 h (Pabst and Binns 1994). This finding hints that lymphocytes are quite fast circulating cells. In swine, a massive recruitment of γδTCR lymphocytes was described after experimental infection with *Actinobacillus pleuropneumoniae* (Faldyna et al. 1995).

The intravascular lymphocyte pool is also of considerable size. Their numbers in pulmonary blood vessels are several times higher than the total number of lymphocytes in the liver or kidney vascular bed (Pabst 1990). The presence of a high number of lymphocytes in pulmonary blood vessels likely does not result from their margination to the pulmonary vessel walls, but is more likely due to a decreased blood flow in pulmonary tissue. This mechanism allows the formation of an available lymphocyte pool. Approximately 1.5×10^9 of lymphocytes are detained in this "depot" in young pigs (Pabst and Binns 1994).

Dunkley et al. (1995) describe a key role played by T lymphocytes in the respiratory tract. They reported that besides the circulation of B lymphocytes activated in Peyer's patches of the gut, there is another recirculation path for activated T lymphocytes. The authors assumed that the colonisation of lungs with T lymphocytes activated in the gut is much more important for the respiratory defence system than the circulation of B lymphocytes.

From the information summarised above it is clear that the respiratory tract is an apparatus whose defence is based on a complicated interplay of mechanical and immunological barriers and mechanisms, and of cellular and humoral factors of innate and specific immunity. Moreover, the action of the "defenders" must be tightly regulated on the border between protection and self-damage.

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Received: 2013–04–09
Accepted after corrections: 2013–04–15

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